

1 We claim:

- 2 1. A method for separating isotopes of an actinide element comprising steps of:
3 providing a composition comprising molecules comprising an actinide
4 element, wherein at least some of the molecules include a first isotope of the actinide
5 element and at least some of the molecules include a second isotope of the actinide
6 element;
7 exposing the molecules comprising the actinide element to reducing activity
8 of actinide element reducing microorganisms, thereby allowing formation of a
9 precipitate comprising the actinide element, wherein the precipitate contains a higher
10 proportion of the second isotope relative to the first isotope than was present in the
11 original composition, thereby effecting a separation of the first and second isotopes;
12 and
13 effecting an increased separation of the first and second isotopes present in
14 the precipitate using any suitable process.
- 15 2. The method of claim 1, wherein the exposing step comprises:
16 combining the composition and the microorganisms in a vessel together with
17 culture medium, thereby producing a culture; and
18 maintaining the culture for a time sufficient to allow formation of a
19 precipitate.
- 20 3. The method of claim 1, further comprising the step of :
21 separating the precipitate from unprecipitated molecules containing the
22 actinide element.
- 23 4. The method of claim 3, wherein the step of separating comprises collecting the
24 precipitate.
- 25 5. The method of claim 3, wherein the step of separating comprises collecting the
26 precipitate and the microorganisms.
- 27 6. The method of claim 5, further comprising the step of treating the microorganisms to
28 remove molecules containing adsorbed, unreduced actinide element atoms
29 therefrom.

- 1 7. The method of claim 1, wherein the exposing step is performed for a time selected to
2 achieve optimum isotope separation.
- 3 8. The method of claim 7, wherein the actinide element is uranium, and the optimum
4 isotope separation results in maximum $\delta^{235}\text{U}$ for the precipitate.
- 5 9. The method of claim 1, wherein the exposing step is performed for a time selected to
6 achieve reduction of less than 80% of the actinide element.
- 7 10. The method of claim 1, wherein the exposing step is performed for a time selected to
8 achieve reduction of less than 60% of the actinide element.
- 9 11. The method of claim 1, wherein the exposing step is performed for a time selected to
10 achieve reduction of less than 40% of the actinide element.
- 11 12. The method of claim 1, wherein the exposing step is performed for a time selected to
12 achieve reduction of less than 20% of the actinide element.
- 13 13. The method of claim 1, wherein the exposing step is performed for a time selected to
14 achieve reduction of less than 10% of the actinide element.
- 15 14. The method of claim 1, wherein the exposing step is performed for a time selected to
16 achieve reduction of less than 5% of the actinide element.
- 17 15. The method of claim 1, wherein the exposing step is performed for a time selected to
18 achieve reduction of less than 1% of the actinide element.
- 19 16. The method of claim 1, wherein the exposing step is performed for a time selected so
20 that at least 20% of the actinide element remains in solution.
- 21 17. The method of claim 1, wherein the exposing step is performed for a time selected so
22 that at least 40% of the actinide element remains in solution.
- 23 18. The method of claim 1, wherein the exposing step is performed for a time selected so
24 that at least 60% of the actinide element remains in solution.
- 25 19. The method of claim 1, wherein the exposing step is performed for a time selected so
26 that at least 80% of the actinide element remains in solution.

- 1 20. The method of claim 1, wherein the exposing step is performed for a time selected so
2 that at least 90% of the actinide element remains in solution.
- 3 21. The method of claim 1, wherein the exposing step is performed for a time selected so
4 that at least 95% of the actinide element remains in solution.
- 5 22. The method of claim 1, further comprising the step of:
6 determining the isotope content of the precipitate.
- 7 23. The method of claim 1, wherein the effecting step comprises:
8 converting the actinide element present in the second composition into a form
9 suitable for repetition of the exposing step; and
10 repeating the exposing step using the converted actinide element as a starting
11 material, thereby effecting an increased separation of the first and second isotopes.
- 12 24. A composition produced according to the method of claim 23.
- 13 25. The composition of claim 24, wherein the actinide element is uranium and the
14 precipitate comprises uraninite.
- 15 26. The method of claim 23, wherein the step of converting comprises:
16 oxidizing at least some of the molecules comprising the actinide element in
17 the precipitate.
- 18 27. The method of claim 26, wherein the oxidizing step is performed by exposing the
19 molecules to ozone, peroxide, other chemical oxidants, or by application of an
20 oxidizing potential.
- 21 28. The method of claim 26, wherein the oxidizing step comprises:
22 exposing the molecules comprising the actinide element in the precipitate to
23 ozone in a solution; and further comprising the step of treating the solution with air
24 to remove the ozone.
- 25 29. The method of claim 26, wherein the oxidizing step comprises:
26 applying an oxidizing potential to the precipitate using an electrode.

- 1 30. The method of claim 26, wherein the step of oxidizing causes solubilization of the
2 oxidized molecules, resulting in a solution suitable for use as a starting material.
- 3 31. A composition produced according to the method of claim 26.
- 4 32. The composition of claim 31, wherein the actinide element is uranium and the
5 precipitate comprises uraninite.
- 6 33. The method of claim 26, further comprising the steps of repeating the method until a
7 desired degree of isotope separation is achieved.
- 8 34. The method of claim 33, wherein the desired degree of isotope separation results in a
9 composition comprising between 3% and 4% ^{235}U .
- 10 35. The method of claim 33, wherein the desired degree of isotope separation results in a
11 composition comprising between 4% and 20% ^{235}U .
- 12 36. The method of claim 23, further comprising the step of :
13 separating the precipitate from unprecipitated molecules containing the
14 actinide element prior to the oxidizing step.
- 15 37. The method of claim 1, wherein the steps are performed in batch mode.
- 16 38. The method of claim 1, wherein the steps are performed in continuous mode.
- 17 39. The method of claim 1, further comprising the step of:
18 desorbing remaining unreduced molecules containing the actinide element
19 from the microorganisms.
- 20 40. The method of claim 1, wherein the microorganisms are present in a medium that is
21 separated from the composition by a semi-permeable membrane during the exposing
22 step, which semi-permeable membrane allows diffusion of the actinide element
23 containing molecules but does not permit passage of the microorganisms.
- 24 41. The method of claim 40, wherein the exposing step comprises allowing the
25 composition to flow continuously past the semi-permeable membrane, thereby

- 1 allowing diffusion of the actinide element containing molecules so as to expose them
2 to the reducing activity of the microorganisms.
- 3 42. The method of claim 41, wherein the exposing step comprises allowing the
4 composition and the medium containing the microorganisms to flow continuously
5 past the semi-permeable membrane, thereby allowing diffusion of the actinide
6 element containing molecules so as to expose them to the reducing activity of the
7 microorganisms.
- 8 43. The method of claim 42, wherein the composition and the medium containing the
9 microorganisms flow in opposite directions.
- 10 44. The method of claim 1, further comprising the step of:
11 immobilizing the microorganisms prior to performing the exposing step.
- 12 45. The method of claim 44, wherein the step of immobilizing the microorganisms
13 comprises:
14 fixing the microorganisms to a solid support.
- 15 46. The method of claim 44, wherein the microorganisms are contained within a semi-
16 permeable membrane or membranes.
- 17 47. The method of claim 44, wherein the exposing step comprises allowing the
18 composition to traverse the immobilized microorganisms.
- 19 48. The method of claim 1, wherein the actinide element is uranium.
- 20 49. The method of claim 48, wherein the precipitate comprises uraninite.
- 21 50. The method of claim 48, wherein the microorganisms reduce uranium from the
22 U(VI) state to the U(IV) state.
- 23 51. The method of claim 48, wherein the first isotope is heavier than the second isotope.
- 24 52. The method of claim 48, wherein the first uranium isotope is U-238.
- 25 53. The method of claim 48, wherein the second uranium isotope is U-235.

- 1 54. The method of claim 48, wherein the first uranium isotope is U-238 and the second
2 uranium isotope is U-235.
- 3 55. The method of claim 1, wherein the method achieves a separation factor of at least
4 1.02.
- 5 56. The method of claim 1, wherein the method achieves a separation factor of at least
6 1.06.
- 7 57. The method of claim 1, wherein the method achieves a separation factor of at least
8 1.10.
- 9 58. The method of claim 1, wherein the actinide element is plutonium.
- 10 59. The method of claim 1, wherein the actinide element is neptunium.
- 11 60. The method of claim 1, wherein the composition comprises an electron donor.
- 12 61. The method of claim 1, wherein the molecules comprising the actinide element are
13 provided together with a counter ion, wherein the counter ion is not an electron
14 accepting species that competes with the actinide element for reduction by the
15 microorganisms.
- 16 62. The method of claim 61, wherein the counter ion can serve as a substrate for
17 metabolism by the microorganisms under anaerobic conditions.
- 18 63. The method of claim 61, wherein the counter ion is selected from the group
19 consisting of: lactate, acetate, and chloride.
- 20 64. The method of claim 1, wherein the microorganisms are metal or sulfate reducing
21 bacteria.
- 22 65. The method of claim 1, wherein the microorganisms are facultative aerobes.
- 23 66. The method of claim 1, wherein the microorganisms are anaerobes.

- 1 67. The method of claim 1, wherein the microorganisms are members of a bacterial
2 genus selected from the group consisting of: *Clostridium*, *Shewanella*, *Geobacter*,
3 *Pyrobaculum*, *Desulfotomaculum*, and *Desulfovibrio*.
- 4 68. The method of claim 67, wherein the microorganisms are members of bacterial
5 genus *Shewanella*.
- 6 69. The method of claim 67, wherein the microorganisms are members of bacterial strain
7 *Shewanella oneidensis*.
- 8 70. The method of claim 67, wherein the microorganisms are selected from the group
9 consisting of *Clostridium* sp., *Deinococcus radiodurans* R1, *Geobacter chapelleii*,
10 *Geobacter hydrogenophilus* H2, *Geobacter hydrogenophilus* H2, *Geobacter* H4,
11 *Geobacter* TACP-2, *Geobacter* TACP-3, *Pyrobaculum islandicum*, *Shewanella alga*,
12 *Shewanella saccharophila*, *Desulfotomaculum reducens* MI-1, *Desulfovibrio*
13 *desulfuricans*, and *Desulfovibrio vulgaris*.
- 14 71. The method of claim 1, wherein the microorganisms are present at a concentration of
15 between approximately 10^7 and 10^9 per milliliter during the exposing step.
- 16 72. The method of claim 1, wherein the microorganisms overexpress a gene encoding a
17 protein that reduces the actinide element.
- 18 73. The method of claim 72, wherein the gene encodes a cytochrome c protein.
- 19 74. The method of claim 73, wherein the cytochrome c is a cytochrome c3.
- 20 75. The method of claim 1, wherein the microorganisms overexpress a gene encoding a
21 protein involved in a pathway leading to reduction of the actinide element.
- 22 76. The method of claim 1, wherein the microorganisms express an altered cytochrome c
23 protein, which altered cytochrome c protein displays an increased ability to reduce
24 the actinide element relative to a wild type version of the protein.
- 25 77. The method of claim 1, wherein the microorganisms express an altered version of a
26 gene encoding a protein involved in a pathway leading to reduction of the actinide

- 1 element, which altered cytochrome c protein displays an increased ability to reduce
2 the actinide element relative to a wild type version of the protein.
- 3 78. The method of claim 1, wherein the exposing step takes place in a medium
4 substantially free of counterions capable of forming insoluble salts with unreduced
5 molecules of the actinide element.
- 6 79. The method of claim 78, wherein the concentration of any counterion capable of
7 forming an insoluble salt with unreduced molecules of the actinide element is less
8 than 10% of the concentration of unreduced molecules of the actinide element in the
9 medium.
- 10 80. The method of claim 78, wherein the concentration of any counterion capable of
11 forming an insoluble salt with unreduced molecules of the actinide element is less
12 than 5% of the concentration of unreduced molecules of the actinide element in the
13 medium.
- 14 81. The method of claim 78, wherein the concentration of any counterion capable of
15 forming an insoluble salt with unreduced molecules of the actinide element is less
16 than 1% of the concentration of unreduced molecules of the actinide element in the
17 medium.
- 18 82. The method of claim 78, wherein the medium lacks significant amounts of
19 phosphate.
- 20 83. The method of claim 1, wherein the exposing step is performed in the presence of an
21 organic polymer.
- 22 84. The method of claim 1, further comprising the step of:
23 culturing the microorganisms under aerobic conditions prior to the
24 maintaining step and performing the maintaining step under anaerobic conditions.
- 25 85. A composition produced according to the method of claim 1.
- 26 86. A method for separating isotopes of an actinide element comprising steps of:

1 providing a composition comprising molecules comprising an actinide
2 element, wherein at least some of the molecules include a first isotope of the actinide
3 element and at least some of the molecules include a second isotope of the actinide
4 element;
5 exposing the molecules comprising the actinide element to reducing activity
6 of actinide element reducing microorganisms, thereby allowing reduction of a
7 portion of the molecules comprising the actinide element to form reduced molecules,
8 wherein the reduced molecules contain a higher proportion of the second isotope
9 relative to the first isotope than was present in the original composition, thereby
10 effecting a separation of the first and second isotopes; and
11 effecting an increased separation of the first and second isotopes present in the
12 reduced molecules using any suitable process.

13 87. The method of claim 86, wherein the actinide element is uranium.

14 88. The method of claim 86, wherein the microorganisms are metal or sulfate reducing
15 bacteria.

16 89. The method of claim 86, wherein the microorganisms are thermophilic bacteria, and
17 wherein the exposing step is performed at a temperature above 50°C.

18 90. The method of claim 86, further comprising the step of:
19 extracting some or all of the reduced molecules from the composition.

20 91. The method of claim 90, further comprising the step of:
21 reextracting the reduced actinide containing molecules into an aqueous phase
22 and either reoxidizing them or collecting them.

23 92. The method of claim 90, wherein the extracting step comprises:
24 forming an extractable organic complex comprising reduced actinide
25 molecules using an organic ligand; and
26 extracting the extractable complex into an organic phase or onto a solid
27 support coated with an organic material.

28 93. The method of claim 92, wherein the organic ligand comprises
29 thenoyltrifluoroacetone.

- 1 94. The method of claim 86, wherein the microorganisms are present in a medium that is
2 separated from the composition by a semi-permeable membrane during the exposing
3 step, which semi-permeable membrane allows diffusion of the actinide element
4 containing molecules but does not permit passage of the microorganisms.
- 5 95. The method of claim 94, wherein the exposing step comprises allowing the
6 composition to flow continuously past the semi-permeable membrane, thereby
7 allowing diffusion of the actinide element containing molecules so as to expose them
8 to the reducing activity of the microorganisms.
- 9 96. The method of claim 94, wherein the exposing step comprises allowing the
10 composition and the medium containing the microorganisms to flow continuously
11 past the semi-permeable membrane, thereby allowing diffusion of the actinide
12 element containing molecules so as to expose them to the reducing activity of the
13 microorganisms.
- 14 97. The method of claim 86, wherein the effecting step comprises:
15 converting the actinide element present in the second composition into a form
16 suitable for repetition of the exposing step; and
17 repeating the exposing step using the converted actinide element as a starting
18 material, thereby effecting an increased separation of the first and second isotopes.
- 19 98. The method of claim 97, wherein the step of converting comprises:
20 oxidizing at least some of the molecules comprising the actinide element in
21 the precipitate.
- 22 99. A composition produced according to the method of claim 86.
- 23 100. The composition of claim 99, wherein the actinide element is uranium.
- 24 101. A method for separating isotopes of an actinide element comprising steps of:
25 providing a composition comprising molecules comprising an actinide
26 element, wherein at least some of the molecules include a first isotope of the actinide
27 element and at least some of the molecules include a second isotope of the actinide
28 element;

1 incubating the molecules comprising the actinide element with an actinide
2 reducing enzyme obtained from an actinide element reducing microorganism,
3 thereby allowing formation of a precipitate comprising the actinide element, wherein
4 the precipitate contains a higher proportion of the second isotope relative to the first
5 isotope than was present in the original composition, thereby effecting a separation
6 of the first and second isotopes; and
7 effecting an increased separation of the first and second isotopes present in
8 the precipitate using any suitable process.

9 102. The method of claim 101, further comprising the step of :
10 separating the precipitate from unprecipitated molecules containing the
11 actinide element.

12 103. The method of claim 102, wherein the step of separating comprises collecting the
13 precipitate.

14 104. The method of claim 101, wherein the exposing step is performed for a time selected
15 to achieve optimum isotope separation.

16 105. The method of claim 101, wherein the actinide element is uranium, and the optimum
17 isotope separation results in maximum $\delta^{235}\text{U}$ for the precipitate.

18 106. The method of claim 101, further comprising the step of:
19 determining the isotope content of the precipitate.

20 107. The method of claim 101, wherein the effecting step comprises:
21 converting the actinide element present in the second composition into a form
22 suitable for repetition of the exposing step; and
23 repeating the exposing step using the converted actinide element as a starting
24 material, thereby effecting an increased separation of the first and second isotopes.

25 108. A composition produced according to the method of claim 107.

26 109. The composition of claim 108, wherein the actinide element is uranium and the
27 precipitate comprises uraninite.

- 1 110. The method of claim 107, wherein the step of converting comprises:
2 oxidizing at least some of the molecules comprising the actinide element in
3 the precipitate.
- 4 111. The method of claim 110, wherein the oxidizing step is performed by exposing the
5 molecules to ozone, peroxide, other chemical oxidants, or by application of an
6 oxidizing potential.
- 7 112. The method of claim 110, wherein the oxidizing step comprises:
8 exposing the molecules comprising the actinide element in the precipitate to
9 ozone in a solution; and further comprising the step of treating the solution with air
10 to remove the ozone.
- 11 113. The method of claim 110, wherein the oxidizing step comprises:
12 applying an oxidizing potential to the precipitate using an electrode.
- 13 114. The method of claim 110, wherein the step of oxidizing causes solubilization of the
14 oxidized molecules, resulting in a solution suitable for use as a starting material.
- 15 115. A composition produced according to the method of claim 110.
- 16 116. The composition of claim 115, wherein the actinide element is uranium and the
17 precipitate comprises uraninite.
- 18 117. The method of claim 110, further comprising the steps of repeating the method until
19 a desired degree of isotope separation is achieved.
- 20 118. The method of claim 117, wherein the desired degree of isotope separation results in
21 a composition comprising between 3% and 4% ²³⁵U.
- 22 119. The method of claim 117, wherein the desired degree of isotope separation results in
23 a composition comprising between 4% and 20% ²³⁵U.
- 24 120. The method of claim 107, further comprising the step of :
25 separating the precipitate from unprecipitated molecules containing the
26 actinide element prior to the oxidizing step.

- 1 121. The method of claim 101, wherein the steps are performed in batch mode.
- 2 122. The method of claim 101, wherein the steps are performed in continuous mode.
- 3 123. The method of claim 101, wherein the enzyme is at least partially purified.
- 4 124. The method of claim 101, wherein an electron donor is present during the incubating
5 step.
- 6 125. The method of claim 101, wherein the actinide reducing enzyme is a cytochrome c
7 enzyme.
- 8 126. The method of claim 125, wherein the cytochrome c is a cytochrome c3.
- 9 127. The method of claim 125, wherein the cytochrome c is at least partially purified.
- 10 128. The method of claim 101, wherein hydrogenase obtained from an actinide element
11 reducing microorganism is present during the incubating step.
- 12 129. The method of claim 128, wherein the hydrogenase is at least partially purified.
- 13 130. The method of claim 128, wherein an electron donor in addition to hydrogenase is
14 present during the incubating step.
- 15 131. The method of claim 101, wherein an electron donor other than hydrogenase is
16 present during the incubating step.
- 17 132. The method of claim 101, wherein a material providing a nucleation site for uraninite
18 formation is provided during the incubating step.
- 19 133. The method of claim 101, wherein the incubating step is performed in a fixed
20 enzyme reactor.
- 21 134. A composition produced according to the method of claim 101.
- 22 135. The composition of claim 134, wherein the actinide element is uranium and the
23 precipitate comprises enriched uraninite.
- 24 136. A method of separating isotopes of an actinide element comprising steps of:

- 1 providing a composition comprising molecules comprising an actinide
2 element, wherein at least some of the molecules include a first isotope of the actinide
3 element and at least some of the molecules include a second isotope of the actinide
4 element;
5 incubating the molecules comprising the actinide element with an actinide
6 reducing enzyme obtained from an actinide element reducing microorganism,
7 thereby allowing reduction of the actinide element to form a reduced actinide
8 element;
9 removing the reduced actinide element;
10 further incubating the reduced actinide element, thereby allowing formation
11 of a precipitate comprising the reduced actinide element, wherein the precipitate
12 contains a higher proportion of the second isotope relative to the first isotope than
13 was present in the original composition, thereby effecting a separation of the first
14 and second isotopes; and
15 effecting an increased separation of the first and second isotopes present in
16 the precipitate using any suitable process.
- 17 137. The method of claim 136, wherein the enzyme is a cytochrome c enzyme.
- 18 138. The method of claim 137, wherein the cytochrome c is a cytochrome c3.
- 19 139. The method of claim 137, wherein the cytochrome c is at least partially purified.
- 20 140. The method of claim 136, wherein hydrogenase obtained from an actinide element
21 reducing microorganism is present during the incubating step.
- 22 141. The method of claim 140, wherein the hydrogenase is at least partially purified.
- 23 142. The method of claim 140, wherein an electron donor in addition to hydrogenase is
24 present during the incubating step.
- 25 143. The method of claim 136, wherein an electron donor other than hydrogenase is
26 present during the incubating step.
- 27 144. A composition produced according to the method of claim 136.

- 1 145. The composition of claim 144, wherein the actinide element is uranium and the
2 precipitate comprises enriched uraninite.
- 3 146. A method for separating isotopes of an actinide element comprising steps of:
4 providing a composition comprising molecules comprising an actinide
5 element, wherein at least some of the molecules include a first isotope of the actinide
6 element and at least some of the molecules include a second isotope of the actinide
7 element;
8 incubating the molecules comprising the actinide element with an actinide
9 reducing enzyme obtained from an actinide element reducing microorganism, ,
10 thereby allowing reduction of a portion of the molecules comprising the actinide
11 element to form reduced molecules, wherein the reduced molecules contain a higher
12 proportion of the second isotope relative to the first isotope than was present in the
13 original composition, thereby effecting a separation of the first and second isotopes;
14 and
15 effecting an increased separation of the first and second isotopes present in
16 the precipitate using any suitable process.
- 17 147. The method of claim 146, wherein the actinide element is uranium.
- 18 148. The method of claim 146, wherein the microorganisms are metal or sulfate reducing
19 bacteria.
- 20 149. The method of claim 146, wherein the microorganisms are thermophilic bacteria, and
21 wherein the exposing step is performed at a temperature above 50°C.
- 22 150. The method of claim 146, further comprising the step of:
23 extracting some or all of the reduced molecules from the composition.
- 24 151. The method of claim 150, further comprising the step of:
25 reextracting the reduced actinide containing molecules into an aqueous phase
26 and either reoxidizing them or collecting them.
- 27 152. The method of claim 150, wherein the extracting step comprises:
28 forming an extractable organic complex comprising reduced actinide
29 molecules using an organic ligand; and

1 extracting the extractable complex into an organic phase or onto a solid
2 support coated with an organic material.

3 153. The method of claim 152, wherein the organic ligand comprises
4 thenoyltrifluoroacetone.

5 154. A composition prepared according to the method of claim 146, wherein the actinide
6 element is uranium.

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